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Phase II Study of Dose-Adjusted EPOCH-Rituximab in Untreated Diffuse Large B-cell Lymphoma with Analysis of Germinal Center and Post-Germinal Center Biomarkers

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Abstract

Purpose—To assess the clinical outcome and the influence of biomarkers associated with apoptosis inhibition (Bcl-2), tumor proliferation (MIB-1) and cellular differentiation on the outcome with dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin and rituximab (DA-EPOCH-R) infusional therapy in diffuse large B-cell lymphoma and analysis of germinal center B-cell (GCB) and post-GCB subtypes by immunohistochemistry.

Patients and Methods—Phase II study of 72 patients with untreated de novo DLBCL who were at least 18 years of age and stage II or higher. Radiation consolidation was not permitted.

Results—Patients had a median age of 50 (range: 19-85) years and 40% had a high-intermediate or high International Prognostic Index (IPI). At five-years, progression-free (PFS) and overall survival (OS) were 79% and 80%, respectively, with a median potential follow-up of 54 months. PFS was 91%, 90%, 67% and 47%, and OS was 100%, 90%, 74% and 37%, for 0-1, 2, 3 and 4-5 IPI factors, respectively, at five-years. The Bcl-2 and MIB-1 biomarkers were not associated with PFS or OS. Based on DA-EPOCH historical controls, rituximab only benefited Bcl-2 positive tumors. Bcl-6 expression was associated with higher PFS whereas GCB exhibited a marginally significant higher PFS compared to post-GCB DLBCL.

Conclusion—DA-EPOCH-R outcome was not affected by tumor proliferation and rituximab appeared to overcome the adverse effect of Bcl-2. Bcl-6 may identify a biological program associated with a superior outcome. Overall, DA-EPOCH-R shows promising outcome in low and intermediate IPI groups. A molecular model of treatment outcome with rituximab and chemotherapy is presented.

INTRODUCTION

CHOP remains the chemotherapy standard for diffuse large B-cell lymphoma (DLBCL) despite modifications over the past 30 years aimed at overcoming drug resistance¹. The basis for these strategies drew from classical concepts which held that non-cross resistant drugs and dose intensity would overcome drug resistance². Evidence today indicates that treatment failure is related to tumor biology on the one hand, and tumor volume, patient condition and pharmacokinetics on the other².

To help address these barriers, we hypothesized that continuous drug exposure may modulate the effects of the cell cycle and apoptosis on treatment response. In this regard, studies have suggested that tumor proliferation is an adverse prognostic factor with bolus regimens such as CHOP and possibly R-CHOP, while in vitro studies suggest that extended drug exposure may enhance cell kill³⁻⁵. This concept formed the basis for Dose-Adjusted (DA)-EPOCH where doxorubicin, vincristine and etoposide are infused over 96 hours⁶. Because the efficacy of infusional schedules are constrained by sub-threshold steady state concentrations (C_{ss}), below which a drug is ineffective, we incorporated pharmacodynamic dosing based on the neutrophil nadir. A phase II study of DA-EPOCH with filgrastim in 50 patients with untreated de novo DLBCL revealed promising results⁶.

The addition of rituximab to CHOP significantly improved the outcome of DLBCL and R-CHOP became the new standard⁷. Several studies, however, suggest the benefit of rituximab varies by tumor pathobiology⁸⁻¹⁰. Two studies found rituximab benefit was primarily in Bcl-2 positive DLBCL, while another study only showed benefit in Bcl-6 negative DLBCL^{8,9}. These biomarkers likely relate to the new molecular taxonomy of DLBCL in which three groups, based on cell of origin, have been defined by gene profiling; the germinal center B-cell (GCB) type, derived from germinal center B-cells, the activated B-cell (ABC) and unspecified (DLBCL_{US}; previously type III) types, derived from post-GCB cells, and the primary mediastinal B-cell (PMBL) type, derived from thymic B-cells¹¹⁻¹³. These groups also have significant differences in clinical features and outcome¹¹⁻¹³. Such findings indicate that DLBCL contains several disease entities which likely have therapeutic implications. To more accurately assess the outcome of rituximab with DA-EPOCH and the role of tumor biology in DLBCL, we present clinical and biomarker results from untreated patients with GCB and post-GCB DLBCL.

PATIENTS AND METHODS

Study Design, Patients and Staging

This is a phase II study of DA-EPOCH-R in untreated de novo GCB and post-GCB DLBCL from the National Cancer Institute (NCI), Massachusetts General Hospital, Boston, and Michael & Dianne Bienes Comprehensive Cancer Center, Holy Cross Hospital, Fort Lauderdale, Florida. The primary objectives were to assess the complete response (CR), survival endpoints and toxicity of DA-EPOCH-R in CD20+ DLBCL, and to assess if Bcl-2 remained a prognostic factor for progression free survival (PFS) as was shown with DA-EPOCH alone⁶. With 33 patients classified as Bcl-2+ (estimated target) and Bcl-2-, the current study would have 79% power to detect a difference between Kaplan-Meier curves according to Bcl-2+ or - expression with 50% and 80% PFS at two years using a one-tailed 0.05 alpha level log-rank test. The actual number of patients enrolled reflects the number of patients with evaluable Bcl-2 immunohistochemistry and the actual ratio of Bcl-2+ and - cases. Based on an informal analysis after enrollment of 35 Bcl-2+ and 24 Bcl-2- cases clearly showing no difference in PFS, further enrollment for this biomarker was terminated. The analysis was restricted to patients with GCB and post-GCB DLBCL, and excluded PMBL due to its distinct pathobiology and infrequent expression of Bcl-2^{6,11,13}. A secondary study objective included analysis of the impact of MIB-1 on clinical outcome. Overall 108 patients were enrolled. Eleven (11%) patients were excluded due to transformed lymphoma in five; discordant lymphoma in two; discordant CD20 expression in one and; non-protocol treatment in three patients. To identify the GCB and post-GCB DLBCL in the 97 eligible/evaluable patients, we excluded the 25 patients with PMBL and report our results in the remaining 72 patients.

All pathology was reviewed by ESJ or SP. Eligibility included stages II-IV, no indolent lymphoma, human immunodeficiency virus negative, negative pregnancy test, and adequate major organ function⁶. The study complied with the Declaration of Helsinki and all patients

gave written informed consent. Initial evaluation included standard blood tests, whole body computed tomography (CT), and bone marrow biopsy. Standard staging and response criteria were employed^{14,15}. Sites of disease were restaged after cycle 4 and every two cycles thereafter.

Chemotherapy and Dose Adjustments

Dose-adjusted EPOCH-R was administered as previously described⁶. Patients received 2 cycles beyond CR or stable changes for a minimum of 6 and a maximum of 8 cycles. Pharmacodynamic dose adjustment was based on twice weekly complete blood counts to achieve limited neutropenia below 500/ μ l⁶. Patients with or at risk of central nervous system disease received intrathecal chemotherapy⁶. Radiation was not permitted on study. Bactrim® DS was administered twice daily for 3 days per week.

Biomarkers

Immunohistochemistry was performed on paraffin-embedded tissue as previously reported³. Sections were stained with monoclonal antibodies to CD20 (clone L26), CD3 (F7.2.38 clone), Bcl-6 (clone PG-B6p), MUM-1 (clone MUM1p), MIB-1 (clone MIB-1) from Dako, Carpinteria, CA, and CD10 (clone 56C6) from Novocastra, Burlingame, CA. Immunohistochemistry was performed at the NCI. For Bcl-2, the staining intensity was compared to the control T cells present in the tumor samples and scored as no tumor cell staining or positive tumor cell staining relative to the T-cells as described. MIB-1 staining was scored as a mean percentile of 200 tumor cells averaged over 3 high power fields. For Bcl-6 and MUM-1, cases were scored as positive if expressed in at least 20% of neoplastic cells. Classification of tumor biopsies into GCB or non-GCB (i.e. post-GCB) subtypes was determined using CD10, Bcl-6, and MUM-1 immunohistochemical markers by the validated method of Hans et al¹⁶.

Statistical Analysis

Survival endpoints were calculated from on-study date until death, relapse, progression, or last follow-up as appropriate. OS, PFS and EFS were calculated using the Kaplan-Meier method and the significance between Kaplan-Meier curves was calculated using the Mantel-Haenszel procedure^{17,18}. Potential follow-up was calculated from study entry to study analysis for each patient. The Cox proportional hazards model was used to identify which factors were jointly significant in the association with OS or PFS¹⁹. Only factors associated with marginal statistical significance or better in a univariate analysis (unadjusted p-value <0.10) were evaluated in a Cox model. All p-values are two-tailed. The analysis of biomarkers was not adjusted for multiple comparisons. MIB-1 and Bcl-2 were prespecified based on hypotheses from the DA-EPOCH study in which these biomarkers were not adjusted, whereas analysis of GCB/post-GCB and Bcl-6 were for hypothesis generation and thus would not require adjustment⁶.

RESULTS

Clinical Characteristics and Outcome

Seventy-two patients with GCB or post-GCB large cell lymphoma were enrolled (Table 1). The patient median age was 50 years (range 19-85) and 40% had a high-intermediate or high International Prognostic Index (IPI)²⁰. Pathological diagnosis included DLBCL in 70 patients and follicular grade 3B in 2 patients.

Of 71 evaluable patients, CR (45) and CR_{unconfirmed} (22) were achieved in 94% and partial response (PR) was achieved in 4% of patients^{14,21}. Among low (0-2) IPI patients, 100%

achieved CR/CR_u compared to 82% in high (3-5) IPI patients. Overall, 15% of patients relapsed including 7% in low and 29% in high IPI and no relapses have yet to be observed beyond 22 months. At five-years, the PFS and OS probability are 79% (95% Confidence Intervals (CI)) (68, 87) and 80% (69, 88), respectively, with a median potential follow-up of 54 months (Figure 1A). When assessed by IPI, the PFS is 91% (72, 98), 90% (71, 97), 67% (42, 85) and 47% (21, 75), and the OS is 100%, 90% (70, 97), 74% (50, 90) and 37% (16, 64) for 0-1, 2, 3, 4-5 IPI risk factors, respectively, at five-years (Figure 1B, 1C). The event-free survival (EFS) is 91% (72, 98), 90% (71, 97), 67% (42, 85) and 31% (13, 58), respectively.

Clinical Prognostic Factors

The IPI was significantly associated with PFS ($p = 0.007$) and OS ($p < 0.0001$) (Figures 1B, 1C) as were the individual IPI risk factors except extranodal sites (data not shown). When assessed in a Cox model, low versus high IPI risk factors was most significantly associated with PFS [$p = 0.006$; Hazard ratio 5.12 (95% CI: 1.61, 16.22)]. For overall survival, however, the final model included age ($>$ versus ≤ 60) [$p = 0.0006$; Hazard ratio 6.46 (95% CI: 2.23, 18.51)] and lactate dehydrogenase (normal versus $>$ normal) [$p = 0.005$; Hazard ratio 18.32 (95% CI: 2.39, 140.39)].

Biological Prognostic Factors

Tumor tissue was analyzed by immunohistochemistry for biomarkers of proliferation (MIB-1), apoptosis inhibition (Bcl-2) and cellular differentiation (CD10, Bcl-6 and Mum-1) (Table 2)^{2,3,16}. As a control group for the effect of rituximab on Bcl-2 PFS, we employed our prior DA-EPOCH study which had identical eligibility and treatment as the present study but did not include PMBL; their exclusion did not significantly alter the outcome (Figure 2B). Clinical risk factors were similar in patients with biomarkers and the entire population (Table 1).

High tumor proliferation was not associated with outcome following DA-EPOCH-R (Table 2; Figure 2A)⁶. We also assessed the effect of rituximab on Bcl-2 associated treatment (Table 1)⁶. While Bcl-2 expression was associated with a worse PFS following DA-EPOCH ($p = 0.03$), as previously reported and now updated at 119 months median follow-up, Bcl-2 had no prognostic impact with DA-EPOCH-R (Table 1; Figure 2B, 2C)^{6,8}.

We examined the outcome of GCB and post-GCB DLBCL with DA-EPOCH-R. Post-GCB tended to be associated with a worse PFS compared to GCB DLBCL (Table 2; Figure 2D)¹⁶. We also examined Bcl-6, a germinal center associated transcription factor. Bcl-6 expression was significantly associated with a higher PFS and OS compared to negative cases (Table 2; Figure 2E). There were insufficient cases with Bcl-6 staining in our prior DA-EPOCH study to provide a historical control.

The prognostic significance of the biomarkers and clinical factors were evaluated in a Cox model. For PFS, the final model included IPI [$p = 0.006$; Hazard ratio 8.98 (95% CI: 1.90, 42)] and Bcl-6 non-expression [$p = 0.066$; Hazard ratio 3.46 (95% CI: 0.91, 13.1)], and for overall survival, the final model included performance status [$p = 0.0007$; Hazard ratio 11.89 (95% CI: 2.83, 49.4)] and Bcl-6 non-expression [$p = 0.0005$; Hazard ratio 12.94 (95% CI: 3.09, 54.1)].

Treatment Dose and Toxicity

Most patients required dose escalations to achieve absolute neutrophil count (ANC) nadirs below 500 with 47% of cycles $\geq 144\%$ (level ≥ 3) above the entry level (Table 3). Treatment was delayed on 2% of cycles. Toxicity was assessed in 72 patients over 414 cycles. ANC nadirs between 100 and 499/ μ l occurred on 38% of cycles and ANC < 100 / μ l on 24% of cycles. Thrombocytopenia below 25,000/ μ l occurred on 9% of cycles. Hospitalization for fever with neutropenia was observed on 19% of cycles. Grade 3 gastrointestinal and neurological

toxicities occurred on 5% of cycles each. No cardiac complications occurred. Three deaths occurred on treatment. One patient died from aspergillus fumigatus infection; one patient died from a subdural hematoma following anticoagulation for a pulmonary embolus and; one patient died at home with presumed sepsis.

DISCUSSION

DA-EPOCH is a unique integration of infusional drug scheduling and pharmacodynamic dosing designed to exploit tumor proliferation and maximize fractional cell kill². Based on its promising efficacy in a phase II study, we conducted a study of DA-EPOCH with rituximab and provide the first report in GCB and post-GCB DLBCL. Overall, 94% of patients achieved CR/CRu and at five years, the PFS and OS are 79% and 80%, respectively, and reflect the low salvage rate of DA-EPOCH-R treatment failures.

To evaluate the effect of rituximab, we explored differences in outcome between our DA-EPOCH and DA-EPOCH-R studies⁶. All benefit occurred in the low IPI patients where the PFS of DA-EPOCH and DA-EPOCH-R was 79% and 91%, respectively, at 5 years⁶. In contrast, high IPI patients had a similar PFS of 58% and 60%, respectively. Our findings of greatest benefit in low IPI patients, similar to other studies, are consistent with the fractional cell kill hypothesis which postulates that chemotherapy kills a fraction of tumor cells based on their relative sensitivity^{2,22-24}. Thus, the likelihood of eradicating all tumor cells would be stochastically determined by the fractional cell kill and tumor volume given a fixed number of cycles. Because the relative impact of increased tumor sensitivity on cure, as might be conferred by rituximab, would be greatest in smaller volume tumors, low risk patients who generally have less disease should benefit most.

While these results suggest DA-EPOCH-R may represent a therapeutic advance, it is difficult to find equivalent R-CHOP studies for reference. A study of R-CHOP by Vose et al, included follicular or marginal zone lymphomas and at least 46% harbored t(14;18)²⁵. A study of dose-dense R-CHOP had limited 20 month median follow-up, and two relatively large reports of R-CHOP were retrospective²⁶⁻²⁸. Of three prospective randomized phase III studies of CHOP versus R-CHOP, the GELA and US intergroup randomized studies were restricted to elderly patients^{23,29}. The phase III MabThera International Trial (MInT) appears to be the best reference, but was limited to favorable patients ≤ 60 years, low (0-1) age-adjusted IPI, allowed stage I disease > 5 cm and 49% of patients received radiotherapy³⁰. At four years, the estimated EFS was 75% for the MInT study compared to 91% and 90% at five years for DA-EPOCH-R in low and low-intermediate IPI patients, respectively. The lower survival of our high IPI patients was in part due to four deaths without progression, and a recent study showed good outcomes with DA-EPOCH-R in high risk patients³¹.

Biomarkers were analyzed to help understand the biological basis of treatment outcome. Tumor proliferation was not associated with outcome, affirming our hypothesis that infusional schedules may avert the adverse effect of high proliferation, a finding we also observed with DA-EPOCH^{5,6}. We also did not find Bcl-2 expression to be prognostic with DA-EPOCH-R, unlike our results with DA-EPOCH. Interestingly, when we compare our two studies, only patients with Bcl-2 positive DLBCL benefited from rituximab, similar to an analysis of Bcl-2 in the GELA study, and suggests biological specificity for rituximab benefit^{8,9,32}. While Bcl-2 is overexpressed in some GCB DLBCL due to t(14;18), expression is highest in ABC DLBCL (a post-GCB subset) due to NF κ B or amplification and carries a poor prognosis^{6,13,33}. These observations suggest Bcl-2 may be a biomarker for a post-GCB DLBCL subset and if so, rituximab might overcome its poor prognosis^{6,32,33}. In support of this hypothesis is a retrospective study by Nyman et al where they showed that the addition of rituximab to CHOP overcame the unfavorable outcome of non-GCB (i.e. post-GCB) DLBCL but did not benefit

GCB DLBCL²⁸. Thus, with R-CHOP, GCB and post-GCB DLBCL showed similar outcomes with an EFS of 68% and 63%, respectively, at 27 months.

Our biomarker analysis suggests GCB may be more favorable than post-GCB DLBCL with DA-EPOCH-R. To help further address this, we assessed Bcl-6 which is overexpressed in most GCB but only a minority of post-GCB DLBCL³⁴. We undertook this analysis based on the US intergroup study R-CHOP versus CHOP which showed that rituximab significantly improved the EFS (64% versus 9%, respectively) of Bcl-6 negative but not Bcl-6 positive DLBCL (40% and 54%, respectively) at 3 years¹⁰. Recently, this group also analyzed p21, which is negatively regulated by Bcl-6, and showed that only p21 positive DLBCL benefited from the addition of rituximab^{35,36}. Taken together, these and other studies provide additional evidence that R-CHOP compared to CHOP mostly benefits post-GCB and obviates the prognostic difference among these biomarkers³⁷. In our study, however, Bcl-6 positive DLBCL fared significantly better than Bcl-6 negative tumors. The excellent outcome of DA-EPOCH-R in Bcl-6 positive or GCB DLBCL, where rituximab appears to have a less important role, suggests it may be superior to R-CHOP in this biological group^{10,28}. When interpreting the meaning of biomarkers, however, it is important to recognize the limitations of immunohistochemistry. Indeed, a recent study concluded that while semiquantitative immunohistochemistry of biomarkers in DLBCL is feasible, their interpretation may be significantly affected by methodology and inter-reader reproducibility³⁸.

These findings raise an hypothesis on the biology of rituximab and chemotherapy treatment outcome (Figure 3). On a molecular level, gene expression signatures of ABC (post-GCB) DLBCL and tumor proliferation are associated with unfavorable survival following CHOP¹³. Biologically, this may be related to the constitutive activation of NFκ-B and its anti-apoptotic target genes, including Bcl-2 and A1^{13,39}. While GCB DLBCL has a better prognosis, many cases are not cured which may relate to the effect of Bcl-6 on cell growth and survival^{35,40}. Mechanistically, the negative regulation by Bcl-6 of cell-cycle arrest genes, p21 and p27Kip1, and DNA damage response genes, p53 and ATR, restrain the apoptotic stress response and promote proliferation, both of which are associated with treatment failure^{3,41,42}. Thus, we propose that outcome of post-GCB DLBCL is primarily constrained by high anti-apoptotic protein levels, whereas GCB DLBCL is constrained by the DNA damage response and high tumor proliferation. Indeed, in some lymphoma cell lines, in vitro treatment with rituximab decreases activated NFκB and its target genes, PRDM1β (positive regulatory domain I) and Bcl-2^{8,32,43,44}. In GCB DLBCL, however, the DNA damage response may be dependent on the chemotherapy regimen^{45,46}. In vitro, the sustained exposure of tumor cells to topoisomerase II inhibition by etoposide and low-dose doxorubicin promote the p53-p21 pathway and activates the check-point kinase (Chk2) independently of ATR, and the cell cycle dependency of topoisomerase II may be advantaged by high proliferation^{45,46}. Such studies indicate that prolonged exposure to topoisomerase II inhibition, as achieved in DA-EPOCH-R, may be particularly effective in Bcl-6 positive or GCB DLBCL. Indeed, it is interesting that DA-EPOCH-R is highly active in Burkitt lymphoma, another germinal center lymphoma with myc activation and high proliferation^{47,48}. Definitive evidence for superiority of DA-EPOCH-R over R-CHOP, however, requires a randomized study which is currently ongoing in the Cancer and Leukemia Group B (CALGB) cooperative group in conjunction with microarray analysis.

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A

B

C

Figure 1.

Survival and Progression-Free Survival of All Patients. Outcomes are reported at five years (95% Confidence Interval (CI)). (A) Progression-free survival (PFS) 79% (68, 87). (B) Overall survival (OS) 80% (69, 88). (C) PFS for low, low-intermediate, high-intermediate and high risk IPI 91% (72, 98), 90% (71, 97), 67% (42, 85) and 47% (21, 75), respectively ($p = 0.007$ for global comparison). (D) OS for low, low-intermediate, high-intermediate and high risk IPI 100%, 90% (70, 97), 74% (50, 90) and 37% (16, 64), respectively ($p < 0.0001$ for global comparison).

A

B

C

D

E

Figure 2.

Progression-Free Survival of Patients with Biomarkers. Outcomes are reported at five years. (A) PFS for MIB-1 <80% and ≥80% in DA-EPOCH-R. (B) PFS for Bcl-2 negative and positive patients following DA-EPOCH (PFS at 5 years: Bcl-2 negative 82% (59, 94) vs. positive 50% (25, 75); $p = 0.03$). Exclusion of PMBL patients from this historical cohort did not significantly alter the balance of IPI factors or PFS at 5 years (Bcl-2 negative 91% (62, 98) vs. positive 57% (25, 84); $p = 0.039$). (C) PFS for Bcl-2 negative and Bcl-2 positive, for DA-EPOCH-R. (D) PFS for GCB versus post-GCB for DA-EPOCH-R (E) PFS for Bcl-6 negative and Bcl-6 positive, respectively, for DA-EPOCH-R.

Figure 3.

Model of drug resistance in GCB and post-GCB DLBCL. In germinal center B-cells, Bcl-6 encodes a transcriptional repressor which negatively regulates the cell cycle inhibitors p21 and p27Kip1 and promotes proliferation. Bcl-6 also suppresses the *p53* tumor suppressor gene and ATM, and modulates DNA damage induced apoptosis. Constitutive activation of NF-κB, a transcriptional regulator, in post-germinal center B-cells increases Bcl-2 and A1 and increases the apoptotic threshold. Clinical evidence that rituximab primarily benefits post-GCB DLBCL and inhibits NF-κB in vitro suggests rituximab modulates post-GCB DLBCL drug resistance. In GCB DLBCL, topoisomerase inhibitors can differentially activate DNA damage checkpoints and may be selectively important. Theoretical relative cytotoxic responses [high to low: █ of rituximab, and CHOP and DA-EPOCH with and without rituximab along the continuum from germinal center to post-germinal center DLBCL are shown. Rituximab benefit is primarily in post-GCB DLBCL where it modulates drug resistance and “equalizes” differences among chemotherapy regimens. In GCB DLBCL, DA-EPOCH-R may show an advantage in part due to extended delivery of topoisomerase II agents.

Table 1

Patient Characteristics

Patient Characteristics	DA-EPOCH-R (All Patients)		DA-EPOCH-R (With Biomarkers)		DA-EPOCH (With Biomarkers)		P ¹
	No.	%	No.	%	No.	%	
Total Patients	72	100	63	100	33	100	
Sex							
Male	43	60	40	63	18	55	0.51 ²
Median Age (Range)	50 (19-85)		50 (20-79)		48 (12-88)		0.33 ³
> 60	20	28	16	25	7	21	
Performance Status							
ECOG ≥ 2	19	26	16	25	3	10	0.064 ²
Stage							
III/IV	11/39	69	11/34	71	6/19	76	0.81 ²
LDH							
>Normal	36	50	32	51	23	70	0.086 ²
Extranodal Sites							
≥2	27	38	22	35	12	36	1.0 ²
IPI							
Low (0-1)	22	31	20	32	12	36	0.92 ⁴
Low-Intermediate (2)	21	29	18	29	6	18	
High-Intermediate (3)	16	22	14	22	11	33	
High (4-5)	13	18	11	17	4	12	

¹ Comparison of DA-EPOCH ± Rituximab with biomarkers. Abbreviations: ECOG-Eastern Cooperative Oncology Group; LDH: Lactate dehydrogenase; IPI: International prognostic index.

² by Fisher's exact test.

³ by Wilcoxon rank sum test.

⁴ by Cochran-Armitage trend test.

Table 2

Biomarkers and Outcome for DA-EPOCH-R

Characteristics	No.	%	<i>I</i> PFS @ 5 years % (95% CI)	<i>I</i> OS @ 5 years % (95% CI)	<i>P</i> ²
MIB-1 ³					
< 80%	13	33	69 (42, 87)	92 (67, 99)	
≥ 80%	39	67	81 (66, 91)	81 (66, 91)	0.40
BCL-2					
Negative	24	41	83 (63, 93)	83 (63, 93)	
Positive	35	59	80 (64, 90)	80 (64, 90)	0.72
DLBCL subtype					
GCB	34	67	79 (63, 89)	84 (68, 93)	
Post-GCB	17	33	56 (30, 79)	64 (41, 83)	0.059
BCL-6					
Negative	14	24	51 (21, 80)	50 (24, 76)	
Positive	44	76	84 (70, 90)	90 (77, 96)	0.0002

¹ Kaplan-Meier estimates at 5 years with median potential follow-up of 54 months.

² *P* =two-tailed *p*-value, derived from log-rank test based on Kaplan-Meier curves.

³ MIB-1 was not significantly associated with PFS or OS when analyzed at other cut points or as a continuous variable (data not shown). Abbreviations: PFS: Progression-Free Survival; OS: Overall survival; GCB DLBCL: Germinal Center B-cell diffuse large B-cell lymphoma.

Table 3

Percent of Each Dose Level Administered Over Treatment Cycles

Dose Levels ¹	Cycle Number (Number (n) of Patients) % Patients At Each Dose Level							
	1 (n = 72)	2 (n = 71)	3 (n = 69)	4 (n = 68)	5 (n = 65)	6 (n = 65)	7 (n = 7)	8 (n = 5)
-1 (80%)		7%	6%	7%	6%	6%		
1 (100%)	100%	45%	9%	9%	6%	5%		
2 (120%)		48%	27%	25%	20%	22%		
3 (144%)			57%	34%	33%	37%	71%	80%
4 (173%)				25%	29%	22%	29%	20%
5 (207%)					6%	9%		

¹ All patients begin at dose level 1 (100%). Percent change relative to dose level 1 shown in parentheses.